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## (54) Protein rab3 GEP

(57) The present invention provides a protein Rab3 GEP which is a GDP/GTP exchange protein active on the Rab3 subfamily small G proteins, which has an amino acid sequence of Sequence ID No. 1 or an amino acid sequence substantially the same as that of the Sequence ID No. 1, a cDNA sequence coding this protein, and genomic DNA sequence to which the cDNA sequence or a part thereof hybridizes. According to the invention, there is provided a novel protein (Rab3 GEP) specific for the Rab3 subfamily small G proteins which are involved in intercellular vesicle trafficking, and a genetic material for industrial utilization thereof. This protein is useful, not only for clarification of the molecular mechanism of intracellular vesicle trafficking which is an important cellular event, but also for development of diagnosis, prevention and therapy of neural diseases and the like.

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## Description

### Field of the Invention

The present invention relates to a GDP/GTP exchange protein (GEP) specific for the Rab3 subfamily small GTP-binding proteins (G proteins). More particularly, the present invention relates to the protein Rab3 GEP useful for clarification of a molecular mechanism of intracellular vesicle trafficking essential for maintenance of homeostasis of a living organism, or for diagnosis or development of preventive and therapeutic drugs for neural diseases.

### Description of the Related Art

In a general cell composing a living organism, there exist a number of organelles surrounded by unit membranes, such as endoplasmic reticulum, Golgi complex, lysosome, and endosome, and material transport between these organelles is accomplished by accurate trafficking of vesicles (intracellular vesicle trafficking). For instance, membrane receptors, such as EGF and PDGF receptors, are synthesized on ribosomes and transported to the endoplasmic reticulum membrane from where they are transported to the plasma membrane through the Golgi complex by vesicles. Soluble substances, such as those secreted outside the cell from the plasma membrane, are also transported by vesicles. For instance, hormones and digestive enzymes are synthesized on ribosomes and transported to the endoplasmic reticulum lumen from where they are transported to the plasma membrane. Exocytosis, endocytosis, and transcytosis are performed by intracellular vesicle trafficking. There are two exocytotic pathways: one is a regulated pathway and the other is a constitutive pathway. In the former pathway, in most cases exocytosis is regulated by $Ca^{2+}$. Intracellular vesicle trafficking is also involved in various other cell functions, such as formation of cell polarity, cytokinesis and cell motility. Although intracellular vesicle trafficking is one of the very important cellular events as described above, the molecular mechanism has not as yet been completely clarified. The mechanism of intracellular trafficking clarified so far is as follows.

There are at least four principal mechanisms in intracellular vesicle trafficking: (i) budding of the vesicle from the donor membrane; (ii) targetting of the vesicle to the acceptor membrane; (iii) docking of the vesicle to the acceptor membrane; (iv) fusion of the vesicle with the acceptor membrane. The vesicle trafficking is regulated by the Rab family small G proteins. There are approximately thirty members in the Rab family and each member is located in each membrane compartment and exerts its specific function. The mode of action of the Rab family members in the targetting and docking processes in intracellular vesicle trafficking is as follows: the GDP-bound inactive form of each Rab family member is complexed with Rab GDP dissociation inhibitor (GDI) and remains in the cytoplasm. When it is released from Rab GDI, GEP exerts its action, and the Rab family member is converted to the GTP-bound active form. This GTP-bound form binds to its specific target protein on the vesicle, which is consequently transported to the acceptor membrane. Before or after fusion of the vesicle with the membrane, the GTP-bound form is converted to the GDP-bound form. Once the GDP-bound form is produced on the membrane, it is complexed with Rab GDI and translocated from the membrane to the cytoplasm.

On the other hand, the present inventors have discovered Rab3A as a member of the Rab family small G proteins (J. Biol. Chem., 263:2879-2904, 1998), and revealed that Rab3A plays an important role in $Ca^{2+}$-dependent exocytosis, particularly in neurotransmitter release (Int. Rev. Cytol., 133: 187-230, 1992). They have further found Rab GDI, a regulatory protein of Rab3A (J. Boil. Chem., 265: 2333-2337, 1990) and Rabphilin3A, a target protein of Rab3A (Mol. Cell. Biol., 13: 2061-2068, 1993).

In intracellular vesicle trafficking, as described above, the mode of action of the Rab family members has been clarified, and the research efforts made by the present inventors have permitted specification of regulatory proteins and target proteins of the Rab family members.

However, in order to understand the more detailed mechanism of intracellular vesicle trafficking, it is indispensable to find GEP and GAP specific for each Rab family member or Rab subfamily. At least, no GEP or GAP specific for the Rab3 subfamily members (Rab3A, -B, -C and -D) has not as yet been identified. Two GEPs for Rab3A, Mss4 (Nature, 361: 464-467, 1993) and Rab3A GRF (J. Boil. Chem., 267: 22715-22718, 1992) have been so far found: the former is not specific for the Rab3 subfamily, and the latter has been just partially purified and its primary structure has not been reported.

### Summary of The Invention

The present invention has an object to provide a novel protein (Rab3 GEP) specific for the Rab3 subfamily small G proteins involved in intracellular vesicle trafficking, in a state that the structure (amino acid sequence) and properties thereof have not been clarified.

Another object of the invention is to provide a material for genetic engineering manipulation of this Rab3 GEP.

The invention provides a protein Rab3 GEP, which is a GDP/GTP exchange protein specific for the Rab3 subfamily small G proteins, and comprises the amino acid sequence of Sequence ID No. 1.

Further, the invention provides an animal protein having substantially the same amino acid sequence as that of the Sequence ID No. 1.

In addition, the invention provides a cDNA sequence encoding the amino acid sequence of the Sequence ID No. 1 or an amino acid sequence substantially the same as that of the Sequence ID No. 1.

The invention also provides a genomic DNA sequence to which the cDNA set forth above or a part thereof is hybridized.

According to the invention, there is provided a novel protein (Rab3 GEP) specific for the Rab3 subfamily small G proteins involved in intracellular vesicle trafficking, and a genetic material for industrially utilizing such a protein. This protein is useful not only for clarifying the molecular mechanism of intracellular vesicle trafficking which is an important cellular event, but also for developing diagnosis, prevention and therapy of neural diseases.

## Brief Description of The Drawings

Fig. 1 illustrates the column chromatographies: (A) shows Superdex 200 column chromatography, and (B) shows the second hydroxyapatite column chromatography (○ represents the [3H]GDP bound which is an indicator of Rab3 GEP activity, ---, absorbance at 280 nm, and the lower panels illustrate SDS-polyacrylamide gel electrophoresis (PAGE) analysis with silver staining);

Fig. 2 illustrates the substrate specificity of Rab3 GEP II (A-1, B-1) and Mss4 (A-2, B-2): (A-1, A-2) Rab3A (○), Rab2 (△), Rab5A (◯), Rab10 (△) and Rab11 (□); and (B-1, B-2) Rab3A (○), Rab3B (△), Rab3C (△), Rab3D (◯); and

Fig. 3A illustrates the requirement of Rab3 GEP II (A-1) and Mss4 (A-2) for lipid modifications of Rab3A , representing their activity to lipid-modified Rab3A (○) and lipid-unmodified Rab3A (◯); and Fig. 3B illustrates the sensitivity of Rab3 GEP II and Mss4 to Rab GDI (with Rab3 GEP II (○), with Mss4 (△), without Rab3 GEP II or Mss4 (◯))

## Detailed Description of The Invention

A protein Rab3 GEP of the invention is purified from rat brain synaptic soluble fraction through successive column chromatographies by using lipid-modified Rab3A as a substrate, and has a molecular weight of about 200 kD as estimated by SDS-PAGE (about 270 kD as estimated by gel filtration). A cDNA clone was obtained from a rat cDNA library with partial amino acid sequences of this purified protein as probes, and the cDNA amino acid sequence was analyzed. This protein Rab3 GEP was confirmed to have the amino acid sequence of Sequence ID No. 1.

Therefore, Rab3 GEP of the invention is available by inserting the foregoing cDNA into an appropriate expression vector, and expressing the cDNA in Escherichia coli and the like. A protein derived from other animals than rat can be obtained by a process, for example, of isolating a cDNA from the cDNA library of the animal by using the cDNA of the invention or a part thereof as a probe, and causing expression in a suitable host-vector system. The thus obtained protein derived from an animal other than rat also has an amino acid sequence substantially the same as that of Sequence ID No. 1.

The cDNA sequence of the invention includes, as described above, cDNA of rat or cDNA coming from any animal other than rat. The genomic DNA sequence of the invention include DNA sequence of any of all the animal species.

## Examples

A protein Rab3 GEP of the invention will be described further in detail by means of Examples. The invention is not however limited by the following examples.

### Example 1: Purification of Rab3 GEP

Synaptic soluble fraction was prepared from 80 rat brains. A half of the fraction (500 ml, 455 mg of protein) was adjusted to 0.2 M NaCl and applied to a Q-Sepharose FF column (2.6 x 10 cm) equilibrated with Buffer A (20 mM Tris/Cl at pH 7.5 and 1 mM DTT) containing 0.2 M NaCl. Elution was performed with 350 ml of Buffer A containing 0.5 M NaCl. Fractions of 10 ml each were collected. When the Rab3 GEP activity was assayed by measuring the dissociation of [$^3$H]GDP from lipid-modified Rab3A, the activity was observed in Fractions 5-19.

These fractions (150 ml, 159 mg of protein) were collected, and NaCl was added to give a final concentration of 2 M. The sample was applied to a phenyl-Sepharose column (2.6 x 10 cm) equilibrated with Buffer A containing 2 M NaCl. Elution was performed with a 360-ml linear gradient of NaCl (2-0 M) in Buffer A, followed by 180 ml of Buffer A. Fractions of 6 ml each were collected. The Rab3 GEP activity was observed in Fractions 52-63.

## Sequence Listing

Sequence ID No.:1

Length:1602 amino acids

Type: protein

Sequence

```
Met Val Gln Lys Lys Phe Cys Pro Arg Leu Leu Asp Tyr Leu Val Ile
 1               5                  10                  15
Val Gly Ala Arg His Pro Ser Ser Asp Ser Val Ala Gln Thr Pro Glu
            20                  25                  30
Leu Leu Arg Arg Tyr Pro Leu Glu Asp His Pro Glu Phe Pro Leu Pro
        35                  40                  45
Pro Asp Val Val Phe Phe Cys Gln Pro Glu Gly Cys Leu Ser Val Arg
    50                  55                  60
Gln Arg Arg Met Ser Leu Arg Asp Asp Thr Ser Phe Val Phe Thr Leu
65                  70                  75                  80
Thr Asp Lys Asp Thr Gly Val Thr Arg Tyr Gly Ile Cys Val Asn Phe
                85                  90                  95
Tyr Arg Ser Phe Gln Lys Arg Met Pro Lys Glu Lys Ala Glu Gly Gly
            100                 105                 110
Ala Gly Pro Arg Gly Lys Glu Gly Ala His Ala Pro Cys Ala Ser Glu
        115                 120                 125
Glu Ala Ala Thr Glu Ser Ser Glu Ser Gly Ser Thr Leu Gln Pro Pro
    130                 135                 140
Ser Ala Asp Ser Thr Pro Asp Val Asn Gln Ser Pro Arg Gly Lys Arg
145                 150                 155                 160
Arg Ala Lys Ala Gly Asn Arg Ser Arg Asn Ser Thr Leu Thr Ser Leu
                165                 170                 175
Cys Val Leu Ser His Tyr Pro Phe Phe Ser Thr Phe Arg Glu Cys Leu
            180                 185                 190
Tyr Thr Leu Lys Arg Leu Val Asp Cys Cys Ser Glu Arg Leu Leu Gly
```

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                195                 200                 205
Lys Lys Pro Gly Ile Pro Arg Gly Val Gln Arg Asp Thr Met Trp Arg
    210                 215                 220
Ile Phe Thr Gly Ser Leu Leu Val Glu Glu Lys Ser Ser Ala Leu Leu
225                 230                 235                 240
His Asp Leu Arg Glu Ile Glu Ala Trp Ile Tyr Arg Leu Leu Arg Ser
                245                 250                 255
Pro Val Pro Val Ser Gly Gln Lys Arg Val Asp Ile Glu Val Leu Pro
            260                 265                 270
Gln Glu Val Gln Gln Ala Leu Thr Phe Ala Leu Pro Asp Pro Ser Arg
        275                 280                 285
Phe Thr Leu Val Asp Phe Pro Leu His Leu Pro Leu Glu Leu Leu Gly
    290                 295                 300
Val Asp Ala Cys Leu Gln Val Leu Thr Cys Ile Leu Leu Glu His Lys
305                 310                 315                 320
Val Val Leu Gln Ser Arg Asp Tyr Asn Ala Leu Ser Met Ser Val Met
                325                 330                 335
Ala Phe Val Ala Met Ile Tyr Pro Leu Glu Tyr Met Phe Pro Val Ile
            340                 345                 350
Pro Leu Leu Pro Thr Cys Met Ala Ser Ala Glu Gln Leu Leu Leu Ala
        355                 360                 365
Pro Thr Pro Tyr Ile Ile Gly Val Pro Ala Ser Phe Phe Leu Tyr Lys
    370                 375                 380
Leu Asp Phe Lys Met Pro Asp Asp Val Trp Leu Val Asp Leu Asp Ser
385                 390                 395                 400
Asn Arg Val Ile Ala Pro Thr Asn Ala Glu Val Leu Pro Ile Leu Pro
                405                 410                 415
Glu Pro Glu Ser Leu Glu Leu Lys Lys His Leu Lys Gln Ala Leu Ala
            420                 425                 430
Ser Met Ser Leu Asn Thr Gln Pro Ile Leu Asn Leu Glu Lys Phe His
```

435 440 445

Glu Gly Gln Glu Thr Pro Leu Leu Leu Gly Arg Phe Ser Asn Asp Leu

450 455 460

Gln Ser Thr Pro Ser Thr Glu Phe Asn Pro Leu Ile Tyr Gly Asn Asp

465 470 475 480

Val Asp Ser Val Asp Val Ala Thr Arg Val Ala Met Val Arg Phe Phe

485 490 495

Asn Ser Ala Asn Val Leu Gln Gly Phe Gln Met His Thr Arg Thr Leu

500 505 510

Arg Leu Phe Pro Arg Pro Val Val Ala Phe Gln Ala Gly Ser Phe Leu

515 520 525

Ala Ser Arg Pro Arg Gln Thr Pro Phe Ala Glu Lys Leu Ala Arg Thr

530 535 540

Gln Ala Val Glu Tyr Phe Gly Glu Trp Ile Leu Asn Pro Ser Asn Tyr

545 550 555 560

Ala Phe Gln Arg Ile His Asn Asn Thr Phe Asp Pro Ala Leu Ile Gly

565 570 575

Asp Lys Pro Lys Trp Tyr Ala His Gln Leu Gln Pro Ile His Tyr Arg

580 585 590

Val Tyr Asp Ser Asn Ser Gln Leu Ala Glu Ala Leu Ser Val Pro Pro

595 600 605

Glu Arg Asp Ser Glu Ser Asp Pro Thr Asp Asp Ser Gly Ser Asp Ser

610 615 620

Met Asp Tyr Asp Asp Ser Ser Ser Ser Tyr Ser Ser Leu Gly Asp Phe

625 630 635 640

Val Ser Glu Met Met Lys Cys Asp Ile Asn Gly Asp Thr Pro Asn Val

645 650 655

Asp Pro Leu Thr His Ala Ala Leu Gly Asp Ala Ser Glu Val Glu Ile

660 665 670

Asp Glu Leu Gln Pro Gln Lys Glu Gly Glu Glu Pro Gly Pro Asp Ser

675 680 685

Glu Asn Ser Gln Glu Asn Leu Pro Leu Arg Ser Ser Ser Ser Thr Thr

690 695 700

Ala Ser Ser Ser Pro Ser Thr Ile Val His Gly Ala His Ser Glu Pro

705 710 715 720

Ala Asp Ser Thr Glu Val Gly Asp Lys Ala Ala Thr Gly Ile Ser Lys

725 730 735

Pro Leu Pro Pro Val Pro Pro Ser Ile Cys Lys Ser Thr Val Asp Arg

740 745 750

Arg Gln Thr Glu Thr Gly Glu Gly Ser Val Cys Gln Arg Thr Tyr Asp

755 760 765

His Pro Tyr Phe Glu Pro Gln Tyr Gly Ser Pro Ala Glu Glu Asp Asp

770 775 780

Asp Glu Gln Gly Glu Ser Tyr Thr Pro Arg Phe Ser Gln His Ala Ser

785 790 795 800

Gly Ser Arg Ala Gln Lys Leu Leu Arg Pro Asn Ser Leu Lys Leu Ala

805 810 815

Ser Asp Ser Asp Ala Glu Ser Asp Ser Arg Ala Ser Ser Pro Asn Ser

820 825 830

Thr Val Ser Asn Asn Ser Thr Glu Gly Phe Gly Gly Ile Met Ser Phe

835 840 845

Ala Ser Ser Leu Tyr Arg Asn His Ser Thr Ser Phe Ser Leu Ser Asn

850 855 860

Leu Thr Leu Pro Thr Lys Gly Ala Arg Glu Lys Thr Thr Pro Phe Pro

865 870 875 880

Ser Leu Lys Gly Asn Arg Arg Ala Leu Val Asp Gln Lys Ser Ser Val

885 890 895

Ile Lys His Ser Pro Thr Val Lys Arg Glu Pro Pro Ser Pro Gln Gly

900 905 910

Arg Ser Ser Asn Ser Ser Glu Asn Gln Gln Phe Leu Lys Glu Val Val

915 920 925

His Ser Val Leu Asp Gly Gln Gly Val Gly Trp Leu Asn Met Lys Lys

930 935 940

Val Arg Arg Leu Leu Glu Ser Glu Gln Leu Arg Val Phe Val Leu Ser

945 950 955 960

Lys Leu Ser Arg Ala Val Gln Ser Glu Asp Asp Ala Arg Gln Asp Val

965 970 975

Ile Gln Asp Val Glu Ile Ser Arg Lys Val Tyr Lys Gly Met Leu Asp

980 985 990

Leu Leu Lys Cys Thr Val Leu Ser Leu Glu Gln Ser Tyr Ala His Ala

995 1000 1005

Gly Leu Gly Gly Met Ala Ser Ile Phe Gly Leu Leu Glu Ile Ala Gln

1010 1015 1020

Thr His Tyr Tyr Ser Lys Glu Pro Asp Lys Arg Lys Arg Ser Pro Thr

1025 1030 1035 1040

Glu Asn Val Asn Thr Pro Val Gly Lys Asp Pro Gly Leu Ala Gly Arg

1045 1050 1055

Gly Asp Pro Lys Ala Met Ala Gln Leu Arg Val Pro Gln Leu Gly Pro

1060 1065 1070

Arg Ala Pro Ser Ala Thr Gly Arg Gly Pro Lys Glu Leu Asp Thr Arg

1075 1080 1085

Ser Leu Lys Glu Glu Asn Phe Val Ala Ser Val Gly Pro Glu Val Ile

1090 1095 1100

Lys Pro Val Phe Asp Leu Gly Glu Thr Glu Glu Lys Lys Ser Gln Ile

1105 1110 1115 1120

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1125 1130 1135

Gln Asp Ser Val Ile Gly Val Ser Pro Ala Val Met Ile Arg Ser Ser

1140 1145 1150

Ser Gln Asp Ser Glu Val Ser Asn Ser Ser Gly Glu Thr Leu Gly Ala

1155 1160 1165

Asp Ser Asp Leu Ser Ser Asn Ala Gly Asp Gly Pro Gly Gly Glu Gly

1170 1175 1180

Ser Ala His Leu Ala Ser Ser Arg Ala Thr Leu Ser Asp Ser Glu Ile

1185 1190 1195 1200

Glu Thr Asn Ser Ala Thr Ser Thr Ile Phe Gly Lys Ala His Ser Leu

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1220 1225 1230

Val Ser Gln Arg Val Tyr Leu Tyr Glu Gly Leu Leu Gly Arg Asp Lys

1235 1240 1245

Gly Ser Met Trp Asp Gln Leu Glu Asp Ala Ala Met Glu Thr Phe Ser

1250 1255 1260

Ile Ser Lys Glu Arg Ser Thr Leu Trp Asp Gln Met Gln Phe Trp Glu

1265 1270 1275 1280

Asp Ala Phe Leu Asp Ala Val Met Leu Glu Arg Glu Gly Met Gly Met

1285 1290 1295

Asp Gln Gly Pro Gln Glu Met Ile Asp Arg Tyr Leu Ser Leu Gly Glu

1300 1305 1310

His Asp Arg Lys Arg Leu Glu Asp Asp Glu Asp Arg Leu Leu Ala Thr

1315 1320 1325

Leu Leu His Asn Leu Ile Ser Tyr Met Leu Leu Met Lys Val Asn Lys

1330 1335 1340

Asn Asp Ile Arg Lys Lys Val Arg Arg Leu Met Gly Lys Ser His Val

1345 1350 1355 1360

Gly Leu Val Tyr Ser Gln Gln Ile Asn Glu Val Leu Asp Gln Leu Thr

1365 1370 1375

Asn Leu Asn Gly Arg Asp Leu Ser Ile Arg Ser Ser Gly Ser Arg His

1380 1385 1390

Met Lys Lys Gln Thr Phe Val Val His Ala Gly Thr Asp Thr Asn Gly

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                1395                1400                1405
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    1410                1415                1420
Asn Ile Gly Thr Val Tyr Glu Arg Trp Trp Tyr Glu Lys Leu Ile Asn
1425                1430                1435                1440
Met Thr Tyr Cys Pro Lys Thr Lys Val Leu Cys Leu Trp Arg Arg Asn
                1445                1450                1455
Gly Ser Glu Thr Gln Leu Asn Lys Phe Tyr Thr Lys Lys Cys Arg Glu
            1460                1465                1470
Leu Tyr Tyr Cys Val Lys Asp Ser Met Glu Arg Ala Ala Ala Arg Gln
        1475                1480                1485
Gln Ser Ile Lys Pro Gly Pro Glu Leu Gly Gly Glu Phe Pro Val Gln
    1490                1495                1500
Asp Met Lys Thr Gly Glu Gly Gly Leu Leu Gln Val Thr Leu Glu Gly
1505                1510                1515                1520
Ile Asn Leu Lys Phe Met His Asn Gln Val Phe Ile Glu Leu Asn His
                1525                1530                1535
Ile Lys Lys Cys Asn Thr Val Arg Gly Val Phe Val Leu Glu Glu Phe
            1540                1545                1550
Val Pro Glu Ile Lys Glu Val Val Ser His Lys Tyr Lys Thr Pro Met
        1555                1560                1565
Ala His Glu Ile Cys Tyr Ser Val Leu Cys Leu Phe Ser Tyr Val Ala
    1570                1575                1580
Ala Val Arg Ser Ser Glu Glu Asp Leu Arg Thr Pro Pro Arg Pro Val
1585                1590                1595                1600
Ser Ser
```

## Claims

1. A protein Rab3 GEP, which is a GDP/GTP exchange protein specific for the Rab3 subfamily G proteins, and comprises the amino acid sequence of Sequence ID No. 1.

2. An animal protein having substantially the same amino acid sequence as that of the Sequence ID No. 1.

3. A cDNA sequence coding the amino acid sequence of the Sequence ID No. 1 or an amino acid sequence substantially the same as that of the Sequence ID No. 1.

4. A genomic DNA sequence to which the cDNA sequence of claim 3 or a part thereof is hybridized.

*Fig. 1*

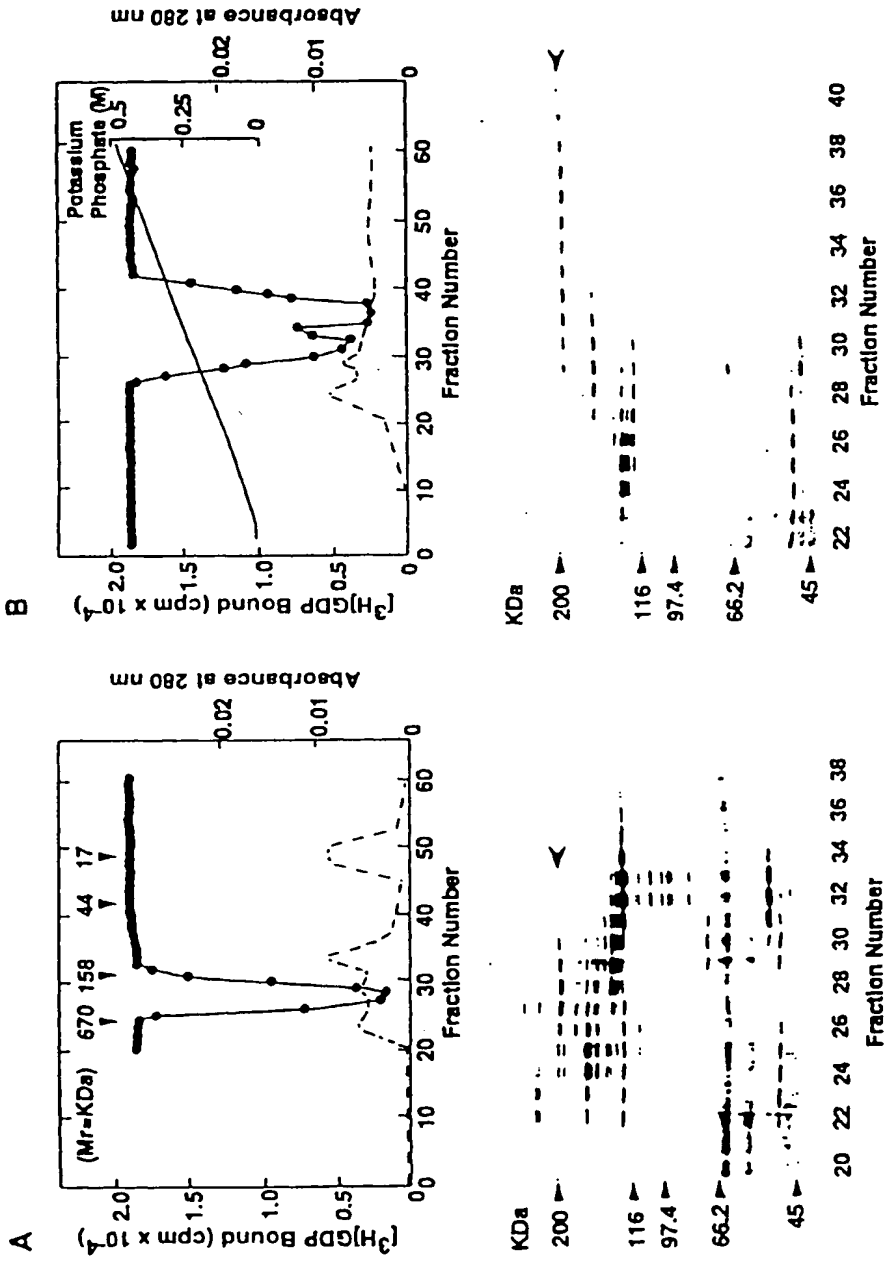
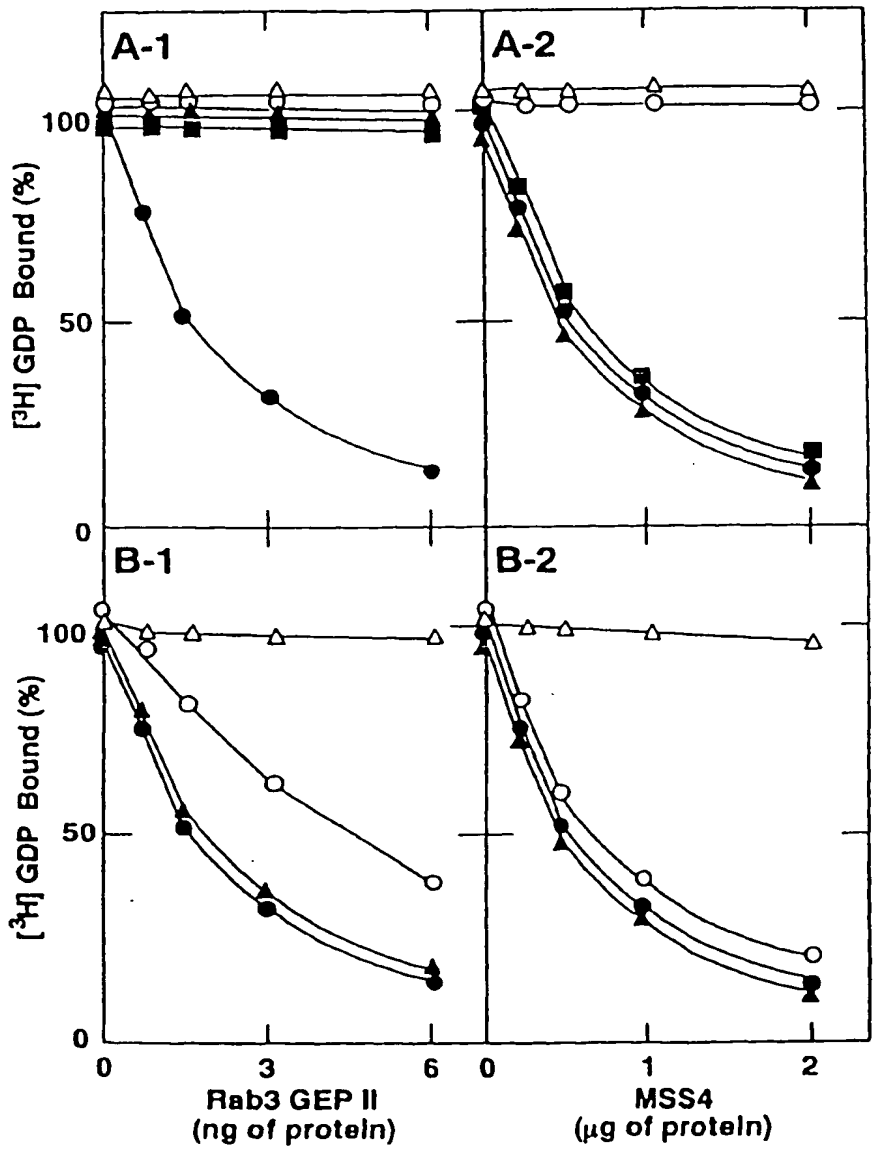

A
B
[3H]GDP Bound (cpm x 10-4)
Absorbance at 280 nm
Fraction Number
(Mr=KDa)
670
158
44
17
Potassium Phosphate (M)
0.5
0.25
0
KDa
200
116
97.4
66.2
45

*Fig. 2*


A-1
A-2
B-1
B-2
100
50
0
[3H] GDP Bound (%)
0
3
6
Rab3 GEP II
(ng of protein)
0
1
2
MSS4
(μg of protein)

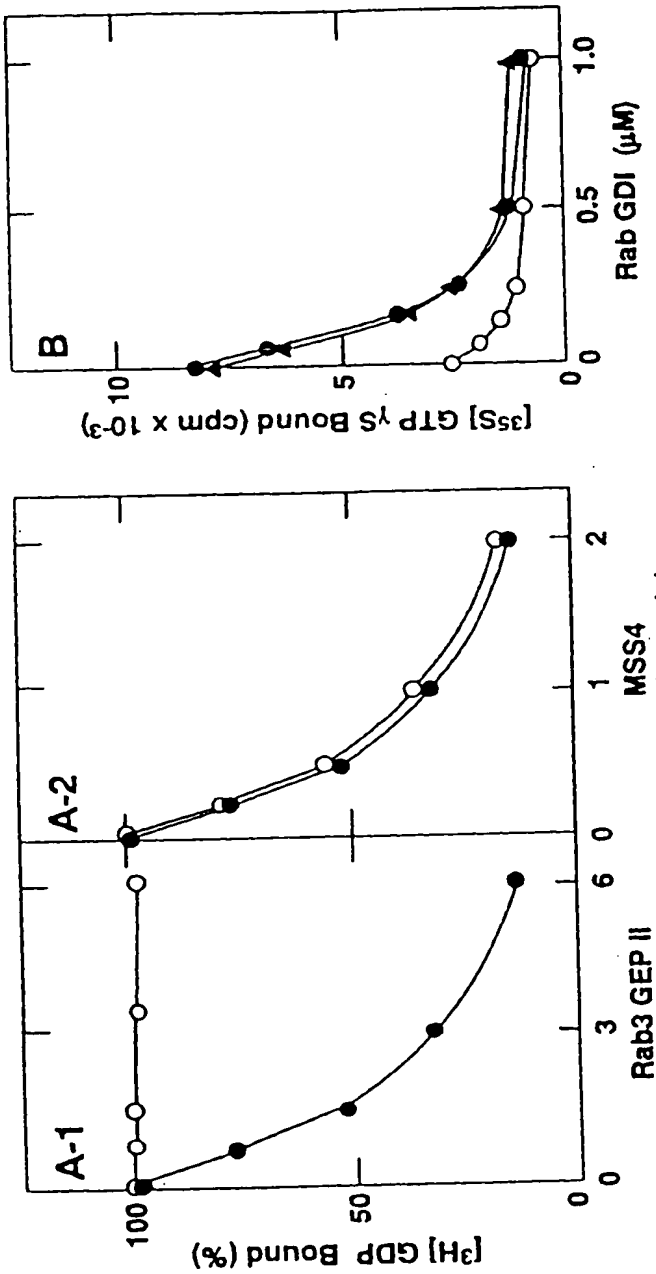

B
[35S] GTP γS Bound (cpm x 10-3)
0
5
10
Rab GDI (μM)
0
0.5
1.0
A-1
[3H] GDP Bound (%)
100
50
0
Rab3 GEP II (ng of protein)
0
3
6
A-2
MSS4 (μg of protein)
0
1
2

*Fig. 3*